

Direct Performance Comparison of Commercially Available Quantitative Quality Control Organisms

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PURPOSE

The purpose of this protocol was to perform a direct comparison of the BioBall™ MultiShot 550, Remel Quanti-CultPlus and the MicroBiologics EZ-Accu Shot™ commercially prepared microbial cultures.

DEFINITIONS

RSD=Relative Standard Deviation

Intra-vial RSD= the variation between aliquot to aliquot of the same vial

Intra-lot RSD= the variation between vials from the same lot

Inter-lot RSD=the variation between different lots of the same product

CofA=Certificate of Analysis

Table 1. Organisms Included in Study.

Organism Name	Culture ID	BTF Part Number	Remel Part Number	MicroBiologics Part Number
<i>Aspergillus brasiliensis</i>	NCPF 2275; ATCC 16404	56011	R4711100	0392A
<i>Bacillus subtilis</i>	NCTC 10400; ATCC 6633	56012	R4711221	0486A
<i>Candida albicans</i>	NCPF 3179; ATCC 10231	56013	R4711503	0442A
<i>Escherichia coli</i>	NCTC 12923; ATCC 8739	56016	R4717085	0483A
<i>Pseudomonas aeruginosa</i>	NCTC 12924; ATCC 9027	56017	R4715210	0484A
<i>Staphylococcus aureus</i>	NCTC 10788; ATCC 6538	56019	R4717016	0485A

Description of Products Included in this Evaluation

• BioBall™ MultiShot 550 consists of a vial that contains a small, freeze-dried sphere containing an average of 500-600 CFU per sphere. When rehydrated with 1.1mL of the BioBall™ rehydration fluid, each vial provides ten aliquots each containing 10-100 organisms per 0.1mL aliquot.

• BioBall™ MultiShot 550 can be rehydrated with rehydration fluid designed for 8 hour stability (BTF Part Number 56021) or with an alternate rehydration fluid which allows the suspension to be frozen and then utilized up to 14 days later (BTF Part Number 410386).

• Remel Quanti-CultPlus consists of a vial which contains a film of organisms attached to the inside cap. When rehydrated with 1.2mL of the Quanti-Cult rehydration fluid, each vial provides ten aliquots each containing <100 organisms per 0.1mL aliquot.

• MicroBiologics EZ-Accu Shot™ consists of a vial containing a lyophilized pellet of a quantified organism population. When rehydrated with 1.2mL of the EZ-Accu Shot™

CONCLUSIONS

Aliquot Counts

• BioBall™ products met the label claim of 10 to 100 cfu/0.1 mL aliquot for all organisms tested at Time Zero and after storage for 8 hours and 14 days.

• Quanti-CultPlus products met the label claim of 1 to 100 cfu/0.1 mL aliquot for all organisms tested at Time Zero and after storage for 8 hours.

• EZ-Accu Shot™ products met the label claim of 1 to 100 cfu/0.1mL for all organisms tested with the exception of two aliquots of *P. aeruginosa* which were >100 cfu/0.1 mL aliquot at Time 0.

• All products tested met label claim for the number of cfu/0.1mL after the hold time specified by the manufacturers, although all products showed some decrease in the overall number of cfu after the hold time compared to Time Zero.

Relative Standard Deviation

• Based on the relative standard deviation (RSD) within and between the different product types, lots and vials the following conclusions can be drawn:

• The Intra-vial RSD (variation between aliquot to aliquot of the same vial) was generally lower for the BioBall™ product, both 14 Day and 8 hours, and the Quanti-CultPlus compared to the EZ-Accu Shot™ products. More of the EZ-Accu Shot™ products exceeded 30% variation from aliquot to aliquot than seen for the other products. This is significant since >30% variation could potentially cause a validation to fail simply due to the variation between aliquots of the challenge organism.

• The Intra-lot RSD (the variation between vials from the same lot) was generally lower for the BioBall™ product, both 14 Day and 8 hours. Significant variation was seen from vial to vial of the EZ-Accu Shot™ products. This can be significant for studies where multiple vials of inoculum are needed to complete a challenge study.

• The Inter-lot RSD (the variation between different lots of the same product) was generally lower for the BioBall™ product, both 14 Day and 8 hours. Significant variation was seen from lot-to-lot of the EZ-Accu Shot™ products.

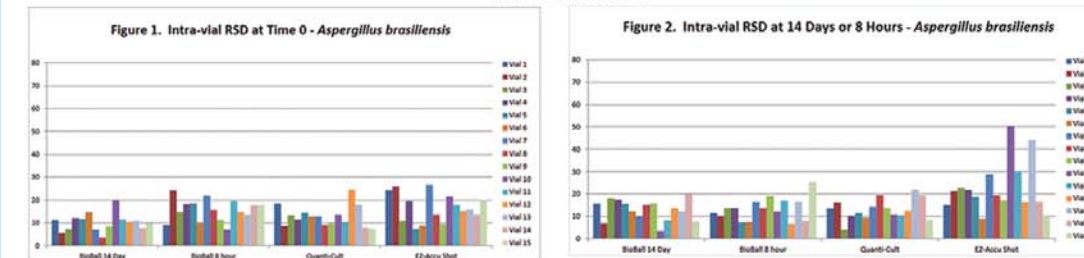
Conformance to Certificate of Analysis

• The Certificate of Analysis (CofA) value for each aliquot of BioBall™ products falls into a very narrow range, 46 – 54 cfu per aliquot. By comparison, the CofA value per aliquot for EZ-Accu Shot™ product ranged from 35 – 73 cfu per aliquot. Remel did not provide an expected vial or aliquot count on Quanti-CultPlus CofAs for these products. The tighter range is an advantage when performing validation studies as it lessens the experimental variation. The tighter range also allows more accurate comparison of verification and validation data between sites and data for studies performed over extended periods of time.

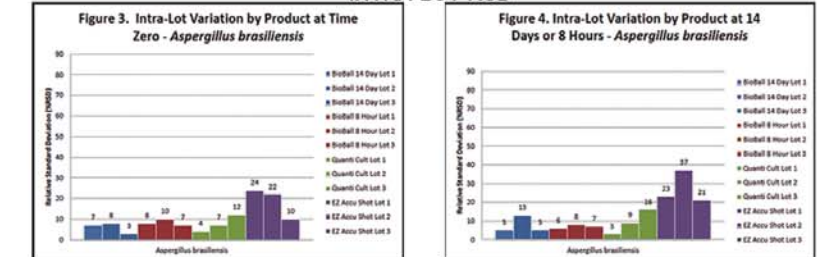
• The lot mean for the BioBall™ product also fell into a narrower range of values than did the Quanti-CultPlus and EZ-Accu Shot™ products, and more closely matched the CofA values than did the EZ-Accu ShotPlus product. Based on this data one can conclude that the BioBall™ product would be most suitable for validation of microbial recovery tests where more consistent recovery rates would give a greater probability of success of the method validation (See figures 7, 14, 21, 28, 35 and 42).

RESULTS FOR ASPERGILLUS BRASILENSIS

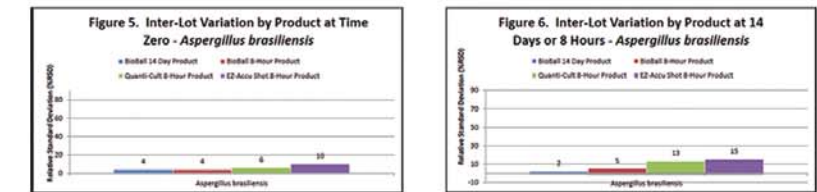
INTRA-VIAL RSD



INTRA-LOT RSD



INTER-LOT RSD



COMPARISON TO CofA

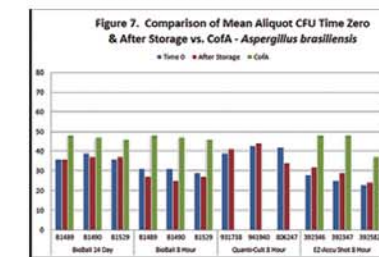


Table 2. Summary of Organism Recovery and Incubation Conditions.

Organism	Recovery Medium	Incubation Time	Incubation Temperature
<i>Aspergillus brasiliensis</i>	Sabouraud Dextrose Agar	3-5 days	20-25°C
<i>Bacillus subtilis</i>	Trypticase Soy Agar	2-3 days	30-35°C
<i>Candida albicans</i>	Sabouraud Dextrose Agar	3-5 days	20-25°C
<i>Escherichia coli</i>	Trypticase Soy Agar	2-3 days	30-35°C
<i>Pseudomonas aeruginosa</i>	Trypticase Soy Agar	2-3 days	30-35°C
<i>Staphylococcus aureus</i>	Trypticase Soy Agar	2-3 days	30-35°C

RESULTS FOR BACILLUS SUBTILIS

INTRA-VIAL RSD

Figure 8. Intra-vial RSD at Time 0 - Bacillus subtilis

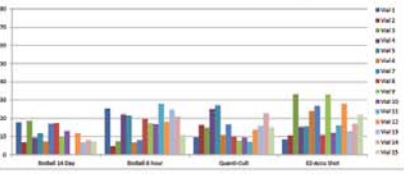
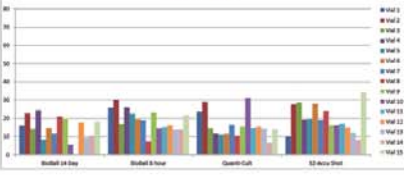


Figure 9. Intra-vial RSD after 14 days or 8 Hours - Bacillus subtilis



INTRA-LOT RSD

Figure 10. Intra-Lot Variation by Product Time Zero - Bacillus subtilis

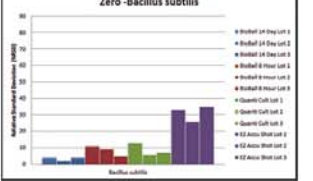
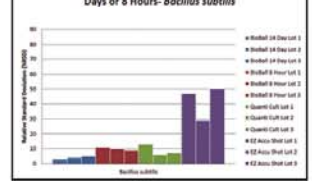


Figure 11. Intra-Lot Variation by Product at 14 Days or 8 Hours - Bacillus subtilis



INTER-LOT RSD

Figure 12. Inter-Lot Variation by Product Time Zero

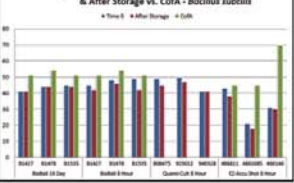


Figure 13. Inter-Lot Variation by Product at 14 Days or 8 Hours



COMPARISON TO CofA

Figure 14. Comparison of Mean Aliquot CFU Time Zero & After Storage vs. CofA - Bacillus subtilis



RESULTS FOR CANDIDA ALBICANS

INTRA-VIAL RSD

Figure 15. Intra-vial RSD at Time 0 - Candida albicans

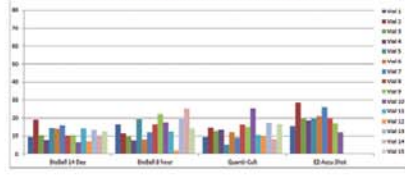
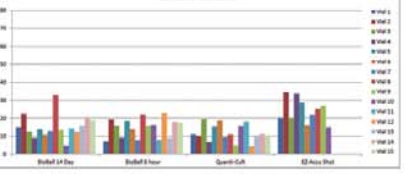


Figure 16. Intra-vial RSD at 14 Days or 8 Hours - Candida albicans



INTRA-LOT RSD

Figure 17. Intra-Lot Variation by Product Time Zero - Candida albicans

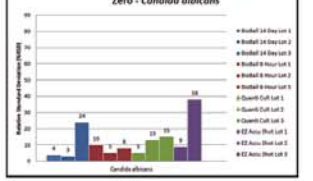
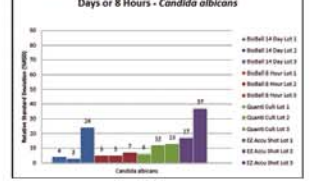


Figure 18. Intra-Lot Variation by Product at 14 Days or 8 Hours - Candida albicans

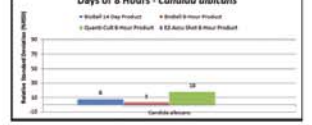


INTER-LOT RSD

Figure 19. Inter-Lot Variation by Product Time Zero - Candida albicans

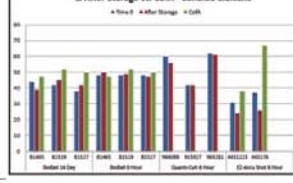


Figure 20. Inter-Lot Variation by Product at 14 Days or 8 Hours - Candida albicans



COMPARISON TO CofA

Figure 21. Comparison of Mean Aliquot CFU Time Zero & After Storage vs. CofA - Candida albicans



RESULTS FOR ESCHERICHIA COLI

INTRA-VIAL RSD

Figure 22. Intra-vial RSD at Time 0 - Escherichia coli

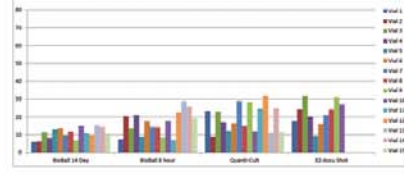
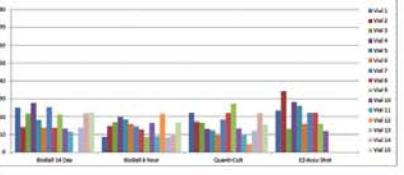


Figure 23. Intra-vial RSD at 14 Days or 8 Hours - Escherichia coli



INTRA-LOT RSD

Figure 24. Intra-Lot Variation by Product Time Zero - Escherichia coli

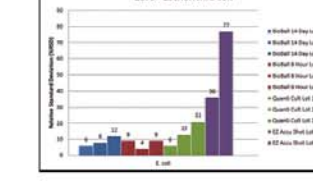
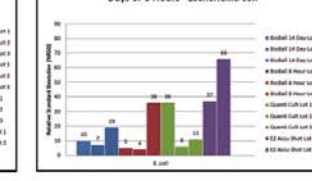


Figure 25. Intra-Lot Variation by Product at 14 Days or 8 Hours - Escherichia coli



INTER-LOT RSD

Figure 26. Inter-Lot Variation by Product at Time Zero - Escherichia coli

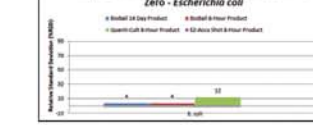
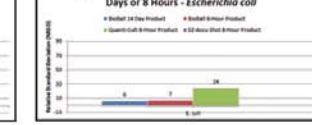
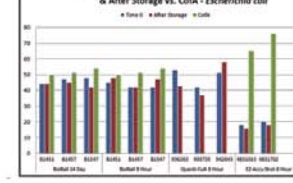


Figure 27. Inter-Lot Variation by Product at 14 Days or 8 Hours - Escherichia coli



COMPARISON TO CofA

Figure 28. Comparison of Mean Aliquot CFU Time Zero & After Storage vs. CofA - Escherichia coli



RESULTS FOR PSEUDOMONAS AERUGINOSA

INTRA-VIAL RSD

Figure 29. Intra-vial RSD at Time 0 - Pseudomonas aeruginosa

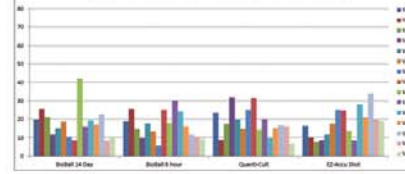
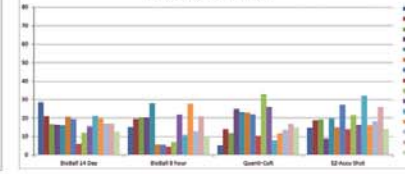


Figure 30. Intra-vial RSD at Time 13 Days or 8 Hours - Pseudomonas aeruginosa



INTRA-LOT RSD

Figure 31. Intra-Lot Variation by Product Time Zero - Pseudomonas aeruginosa

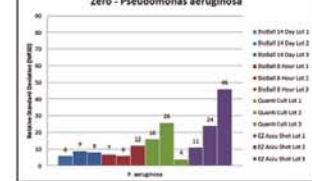
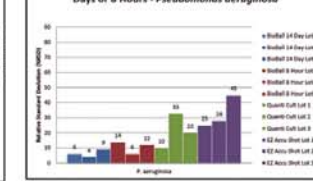


Figure 32. Intra-Lot Variation by Product at 14 Days or 8 Hours - Pseudomonas aeruginosa



INTER-LOT RSD

Figure 33. Inter-Lot Variation by Product Time Zero - Pseudomonas aeruginosa

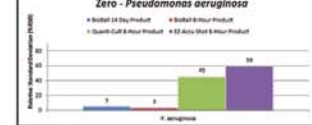
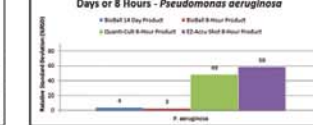
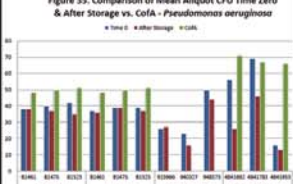


Figure 34. Inter-Lot Variation by Product at 14 Days or 8 Hours - Pseudomonas aeruginosa



COMPARISON TO CofA

Figure 35. Comparison of Mean Aliquot CFU Time Zero & After Storage vs. CofA - Pseudomonas aeruginosa



RESULTS FOR STAPHYLOCOCCUS AUREUS

INTRA-VIAL RSD

Figure 36. Intra-vial RSD at Time 0 - Staphylococcus aureus

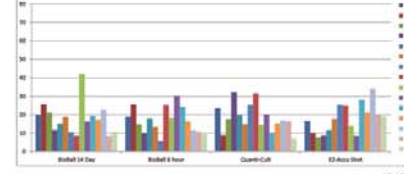
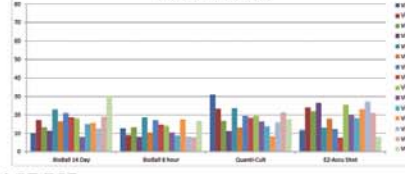


Figure 37. Intra-vial RSD at 14 Days or 8 Hours - Staphylococcus aureus



INTRA-LOT RSD

Figure 38. Intra-Lot Variation by Product Time Zero - Staphylococcus aureus

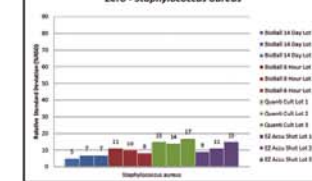
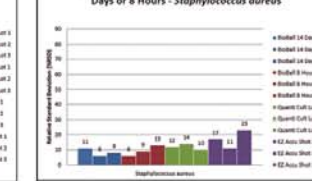


Figure 39. Intra-Lot Variation by Product at 14 Days or 8 Hours - Staphylococcus aureus



INTER-LOT RSD

Figure 40. Inter-Lot Variation by Product Time Zero - Staphylococcus aureus

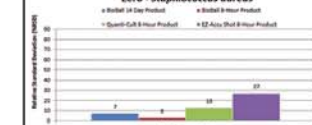
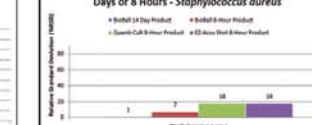
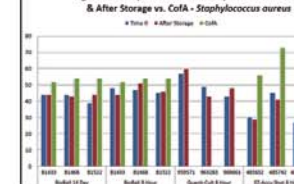


Figure 41. Inter-Lot Variation by Product at 14 Days or 8 Hours - Staphylococcus aureus



COMPARISON TO CofA

Figure 42. Comparison of Mean Aliquot CFU Time Zero & After Storage vs. CofA - Staphylococcus aureus



Acknowledgement:
This study was commissioned by BTF,
a BioMerieux company.